Developmental adaptation of withdrawal reflexes to early alteration of peripheral innervation in the rat

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1. In adult decerebrate spinal rats whose plantar nerves (PLN) had been transected at either postnatal day 1 (P1) or P21 the nociceptive withdrawal reflexes (NWR) of musculi extensor digitorum longus (EDL), peroneus longus (PER) and semitendinosus (ST) were characterized with respect to receptive field (RF) organization, magnitude and time course, using electromyography. Thermal (short CO_2 laser pulses) and mechanical (calibrated pinch) stimulation were used. The innervation patterns in normal and lesioned adult rats were assessed by acute nerve lesions.

2. The spatial organization of the mean mechano- and thermonociceptive RFs of all the muscles studied was similar to normal in both P1- and P21-lesioned rats, although in some P21-lesioned rats atypical EDL RFs were encountered.

3. In P1-lesioned rats thermo-NWR of PER and EDL had normal magnitudes, while mechano-NWR were reduced. In P21-lesioned rats both thermo- and mechano-NWR of these muscles had reduced magnitudes. Except for thermo-NWR of ST in P1-lesioned rats, which were increased, NWR of ST had normal magnitudes in both P1- and P21-lesioned rats. The time course of thermonociceptive NWR of the muscles studied were near normal in both P1- and P21-lesioned rats.

4. Acute nerve lesions in adult P1-lesioned rats revealed an essentially abolished contribution to NWR from the PLN. Instead, the contribution to NWR from other hindpaw nerves, such as the superficial and deep peroneal nerves, was dramatically increased. By contrast, in P21-lesioned rats, the regenerated PLN contributed significantly to the NWR.

5. It is concluded that despite profound alterations of plantar hindpaw innervation induced by early PLN transection the cutaneous nociceptive input to NWR attained an essentially normal spatial organization. An experience-dependent mechanism is suggested to be instrumental in adapting the reflex connectivity to the peripheral innervation.

Adequate sensorimotor transformation depends on highly ordered representations of the body surface in the central nervous system. Following peripheral nerve lesions these representations may be distorted due to alterations of peripheral innervation and central termination patterns of both lesioned and intact afferents (Devor, Schonfeld, Seltzer & Wall, 1979; Horch, 1979; Fitzgerald, 1985; Snow & Wilson, 1989; Shortland & Fitzgerald, 1994). Reinnervation of a denervated skin area depends on the combined actions of collateral sprouting of adjacent intact nerves (Weddell, Guttmann & Gutmann, 1941; Devor *et al.* 1979; Kinnman & Aldskogius, 1986), and regeneration of the lesioned nerves (Devor *et al.* 1979). The peripheral terminals of regenerating afferent fibres rarely reoccupy their former locations (Horch, 1979). Central reorganization induced by nerve lesions includes changes in the laminar projections of afferent fibres (Woolf, Shortland & Coggeshall, 1992; Shortland & Fitzgerald, 1994), and, particularly in young animals, altered somatotopy (Fitzgerald, 1985; Snow & Wilson, 1989). It is not known whether adequate sensorimotor transformation can be established despite these alterations of body surface representation.

The cutaneous withdrawal reflexes of rat hindlimb muscles may be a useful model in the study of this issue since they represent spatially well-organized sensorimotor transformations (Schouenborg & Kalliomäki, 1990; Kalliomäki, Schouenborg & Dickenson, 1992; Schouenborg, Holmberg & Weng, 1992; Schouenborg & Weng, 1994; Holmberg & Schouenborg, 1996; see also Schouenborg, Weng & Holmberg, 1994). These reflexes appear to have a 'modular' organization, each reflex pathway essentially controlling a single muscle or a small group of synergistic muscles. In adult rats, the location and distribution of sensitivity within the cutaneous excitatory receptive field of a reflex pathway directly reflect the withdrawal movement pattern that is caused by the effector muscle(s) in the standing position. For example,

maximal reflex responses in a single muscle are evoked from the skin area which is most effectively withdrawn from the stimulation as the muscle contracts.

In the present study we have examined how altered peripheral innervation patterns during early postnatal life affect the development of the withdrawal reflex system. The medial and lateral plantar nerves, which provide the principal cutaneous innervation of the plantar hindpaw (Swett & Woolf, 1985; Molander & Grant, 1986; Wiesenfeld-Hallin, 1988), were transected at either postnatal day 1 (P1) or at P21, i.e. either before or after the establishment of an adult nociceptive withdrawal reflex organization (Holmberg & Schouenborg, 1996). When the rats had reached adulthood, the withdrawal reflexes of three hindlimb muscles, which normally have excitatory receptive fields on the plantar paw, were examined by characterization of time course, magnitude and receptive field organization. The innervation patterns in normal and lesioned adult rats were assessed by acute nerve lesions.

METHODS

Animals used

Forty-four Wistar rats of both sexes were used in the present study (26 nerve-lesioned rats and 18 normal rats). Supplementary data from normal adult rats were obtained from a previous study in which identical experimental procedures were used ($n = 14$ rats, Holmberg & Schouenborg, 1996). All rats received food and water *ad libitum* and were kept in a 12 h day–12 h night cycle and at a constant environmental temperature of 21 °C (humidity, 65%). Approval for the experiments was obtained in advance from the University of Lund Local Ethical Committee.

Surgery in young rats

Rat pups underwent surgery at either postnatal (P) day 1 (defined as the first 24 h after birth, $n = 17$ rats) or at P21 ($n = 9$ rats). Three or four rat pups from each litter were used. Neonatal rats were anaesthetized by hypothermia (cooling on ice). Rats in the P21 group were anaesthetized by sodium pentobarbitone (35 mg kg^{-1} I.P.). Careful infiltration of the plantar nerves and surrounding tissues of 2·0 mg ml^{-1} lignocaine (lidocaine; Xylocaine) with 1·2 μg ml^{-1} adrenaline was made prior to surgery in order to block nociceptive input and to minimize bleeding. Surgery (which was accomplished within 10–15 min) commenced when there were no spontaneous movements and when no reflexes could be evoked by pinching the skin. The medial and lateral plantar nerves were identified in the right hindlimb through a 4–8 mm-long skin incision medial to the Achilles tendon and transected with a fine pair of scissors 1–3 mm proximal to the medial malleolus. No attempts were made to facilitate or prevent regeneration of the transected nerves. Fine resorbable thread (Vicryl 8-0, GS-9; Ethicon D-22851, Norderstedt, Germany) and Nobecutan wound spray (Astra Tech Inc., Mölndal, Sweden) were used to close skin incisions. Rats in the P1 groups were allowed to recover in a temperature-controlled environment and returned to the home cage after regaining normal body temperature (measured by a non-contact infrared-detecting probe; Thermonitor C-1600M, Linear Laboratories, Los Altos, CA, USA). Rats in the P21 group were returned to the home cage after recovering from anaesthesia. All rats recovered uneventfully and did not exhibit any signs of suffering (such as vocalization, writhing, immobilization or sustained flexion of the manipulated limb) during recovery after surgery. The growth and behaviour of the operated pups were indistinguishable from that of unoperated litter mates (monitored daily in both age groups until the day of the acute experiment), and in no case were there any signs of infection.

To confirm that the denervations were successful, the cutaneous sensibility was tested on the day after surgery by giving five to ten CO_2 laser pulses (Directed Energy Inc., Irvine, CA, USA; intensity 25 mJ, i.e. about twice reflex thresholds for the skin on the plantar surface of the contralateral foot; see also Holmberg & Schouenborg, 1996). In none of the lesioned rats could any reflex responses be evoked on stimulation of the distal and central part of the plantar hindpaw. In normal adult rats a similar loss of cutaneous sensibility occurred after acute transection of the plantar nerves (see Results).

Surgery and preparation in adult rats

The normal rats were investigated 9–14 weeks after birth and the lesioned rats 10–14 weeks after the nerve transection. The rats were anaesthetized with halothane (1·0–2·0%) in a mixture of 65% nitrous oxide and 35% oxygen and were artificially ventilated via a tracheal cannula. The expiratory CO_2 (3·0–4·5%) was monitored continuously. An infusion of 30–50 μl min^{-1} of 5% glucose in Ringer acetate solution (pH 7·0) was given via the right jugular vein. The common carotid arteries were ligated prior to decerebration to reduce bleeding. Mean arterial blood pressure (75–140 mmHg) was monitored continuously in the right carotid artery. The core temperature was kept between 36·5 and 38·5 °C using a thermostatically controlled feedback-regulated heating system. A laminectomy of the tenth thoracic vertebra and a craniotomy were made and the cranial contents rostral to the inferior colliculus were removed by suction. The anaesthesia was then discontinued and the exposed spinal cord transected with a fine pair of scissors. The exposed tissue was moistened with saline (9 mg NaCl (ml water)$^{-1}$). Local infiltration of lignocaine with adrenaline (concentrations as above) was made to reduce nociceptive input during surgery and to minimize possible post-operative excitability changes (Clarke & Matthews, 1990). Immediately following spinalization, a small incision was made in the skin overlying the investigated muscles to facilitate insertion of the electromyography (EMG) needles (see below) into the muscle bellies. Experiments were terminated on signs of deterioration, i.e. precipitate drops in blood pressure or expiratory CO_2 levels. After termination of the experiments the animals were killed by an overdose of halothane (5% for > 15 min). The nerve-lesioned animals were then perfused with 10% formalin and the medial and lateral plantar nerves in both hindlimbs were dissected in order to determine the location of the neuroma. The plantar nerves were saved for later examination.

Electromyography recordings

Reflex responses were recorded with etched fine steel electrodes (insulated up to 50 μm from the tip; diameter at the distal end of insulation, 30–40 μm; tip diameter less than 3 μm; length of the electrode, 14 mm; weight, 5 mg) inserted into the belly of the muscles. Each electrode was soldered to a delicate flexible copper wire (diameter, 0·08 mm). Reference electrodes were placed in the adjacent skin. Up to three muscles were recorded simultaneously. To reduce the risk of contamination of the recordings by responses of nearby muscles, care was taken to ensure that the recording electrodes were placed centrally in the muscle bellies. This was confirmed by electrically stimulating the recorded muscles through the recording electrodes (see Schouenborg *et al.* 1992). In no case was the threshold current needed to activate nearby muscles less than ten times that for the muscle studied.

Mapping of cutaneous excitatory receptive fields

Calibrated noxious pinch and CO_2 laser stimulation of between twenty-five and forty sites on the plantar hindpaw skin were used to map the plantar cutaneous excitatory receptive fields of the extensor digitorum longus (EDL), peroneus longus (PER) and semitendinosus (ST) muscles. Mapping of mechanonociceptive receptive fields started 2 h after spinalization. The flat surface of a calibrated pinching device (1 mm^2 on each side) was applied to a skin flap of about 4 mm^2 and the pinch force was slowly increased (around 1 N s^{-1}) and kept between 2·0 and 2·5 N for more than 1 s (Schouenborg *et al.* 1992). Mapping of thermonociceptive receptive fields with CO_2 laser stimulation (unfocused beam; diameter, 1·1 mm; intensity, 1 W; pulse duration, 20–25 ms, i.e. twice reflex threshold) started 4·5 h after spinalization. Interstimulus intervals were about 1 min during both types of receptive field mapping.

Analysis

The magnitude of the reflex responses was defined as the number of clearly distinguishable motor unit spikes (i.e. spikes with an amplitude exceeding 40 μV which could be separated from background noise) evoked during the first second after the onset of the CO_2 laser pulse or during the first second of constant pinch force (spike numbers were counted using the EGAA program; RC Electronics Inc., Goleta, CA, USA).

To describe the mean receptive field of a muscle in an experimental group, the responses in this muscle, evoked by stimulation of the hindpaw in all rats of this experimental group, were plotted on the corresponding stimulation sites on a standard diagram of the hindpaw. For each muscle in each rat, responses evoked by stimulation of the plantar side of the foot were normalized and expressed as a percentage of the maximal response for the respective muscle and rat. For each stimulated site a mean response value was then calculated. From these mean values, a mean receptive field was constructed which was divided into three areas of differing sensitivity: maximal sensitivity (70–100% of maximal response; referred to as the focus of the receptive field), medium sensitivity (30–70% of maximum) and low sensitivity (< 30% of maximum). The areas of differing sensitivity were delineated with the aid of computer-generated isoresponse lines (Kriging algorithm and contour program, software from Golden Inc., Golden, CO, USA; 'Grid' and 'Topo' programs; see also Schouenborg, Weng, Kalliomäki & Holmberg, 1995).

For the mean receptive field of each of the muscles studied a quantitative comparison of the spatial input–output relationship of the withdrawal reflexes was made between normal rats and rats lesioned at P1 and P21, respectively. The method used for this comparison has previously been described in detail (Ekerot, Garwicz & Schouenborg, 1991; Schouenborg *et al.* 1995). In brief, the mean response values, from which the mean receptive fields were depicted, were used to calculate 'response values' of a dense standard grid of points on the hindpaw (using the Kriging algorithm, see above). The correlation between different mean receptive fields (expressed as the correlation coefficient, r) was then calculated by linear regression analysis between the grid point response values of the respective mean receptive fields.

For each muscle and rat studied, the two strongest reflex responses evoked from the skin area corresponding to the normal adult receptive field foci of the respective muscle were used to compare the reflex response magnitudes (CO_2 laser and noxious pinch stimulation) and latencies (CO_2 laser stimulation) in manipulated rats with those in normal rats.

Mapping of the functional innervation territory of different hindlimb nerves

Cutaneous sensory innervation of the rat hindpaw is provided by the medial and lateral plantar nerves, the common peroneal nerve, the sural nerve and the saphenous nerve (Greene, 1963). The contribution from the plantar nerves, and from the other hindlimb nerves, to PER, EDL and ST reflexes evoked from the plantar surface of the foot was investigated in adult rats lesioned at P1 or P21 and in normal adult rats by acute nerve transections during the terminal experiments.

The plantar nerves were acutely transected proximal to the neuroma at midcalf level (location of the neuroma was determined by dissection). The saphenous nerve was transected at midthigh level, the sural nerve and the superficial branch of the common peroneal nerve just proximal to the ankle, and the deep branch of the common peroneal nerve proximally at the dorsal side of the hindpaw.

In order to reduce possible central effects, local infiltration of 2·0 mg ml^{-1} lignocaine with 1·2 μg ml^{-1} adrenaline was made prior to the acute nerve transections, and a pause of at least 20 min was made before the recordings commenced. All manipulated nerves were dissected after each experiment in order to confirm that the acute nerve transections had been performed as intended.

Statistical analysis

The non-parametric Mann–Whitney U test was used for statistical evaluation. Significant differences were assumed at the level of $P < 0·05$. Values are given as means ± S.E.M.

RESULTS

Receptive fields of nociceptive reflexes

In adult rats lesioned at P1 the mean receptive fields of PER, EDL and ST were highly similar to their counterparts in normal animals. This was the case both for receptive fields mapped with noxious mechanical stimulation ($n = 7$ rats for all three muscles; Fig. 1) and with CO_2 laser stimulation ($n = 5$ rats for all three muscles; Fig. 2). Thus, PER reflexes were evoked from the lateral distal part of the plantar side of the foot, with maximal responses from the skin overlying the distal part of the fifth metatarsal bone and from digit 5. EDL reflexes were evoked from the distal plantar side, with maximal responses from the plantar distal side of the digits. ST reflexes were evoked from most of the paw, with maximal responses from the lateral plantar side. Mechano- and thermonociceptive receptive fields of the muscles studied were similar, although the focus area of the mechanonociceptive receptive field of ST was larger than in normal rats. Correlation coefficients (r) between the mean receptive fields (see Methods) of rats lesioned at P1 and those of normal rats ranged from 0·76 to 0·94.

In adult rats lesioned at P21 the mean PER and ST receptive fields appeared essentially normal, whereas the mean EDL receptive field differed somewhat from its normal counterpart (Figs 1 and 2, bottom rows; noxious pinch and CO_2 laser, respectively). Correlation coefficients (r) between the mean receptive fields of this group of rats and normal rats ranged from 0·48 to 0·92. For EDL, both

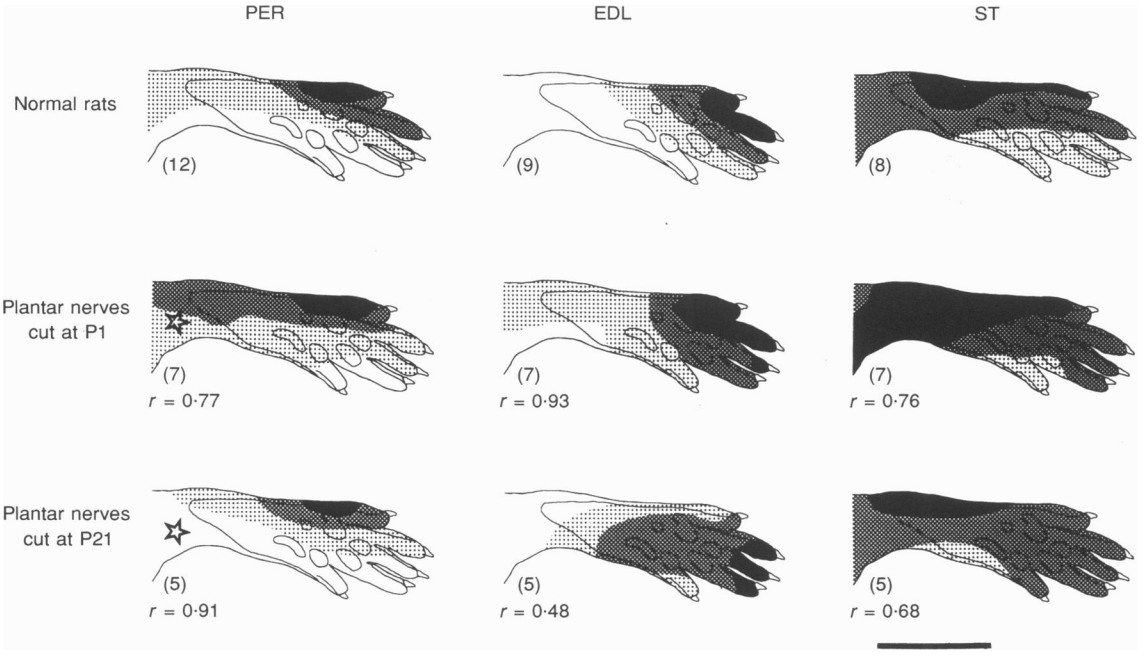

Figure 1. Receptive fields of three hindlimb muscles obtained in normal, P1-lesioned and P21-lesioned rats with noxious mechanical stimulation

Mean receptive fields (see Methods) of peroneus longus (PER), extensor digitorum longus (EDL) and semitendinosus (ST) obtained using noxious mechanical stimulation (calibrated pinch) in normal adult rats (upper row), and in rats subjected to transection of the plantar nerves at P1 or P21 (middle and bottom row, respectively). Low, medium and high density of dots indicate areas of the skin from which the evoked reflexes were 0–30%, 30–70% and 70–100% of maximal response, respectively. Numbers in paretheses indicate n, the number of rats. For each muscle the correlation coefficient (r; see Methods) between the mean receptive field obtained in lesioned and normal rats is indicated. Scale bar, 20 mm. ☆, site of the neuroma (location determined by post-mortem dissection).

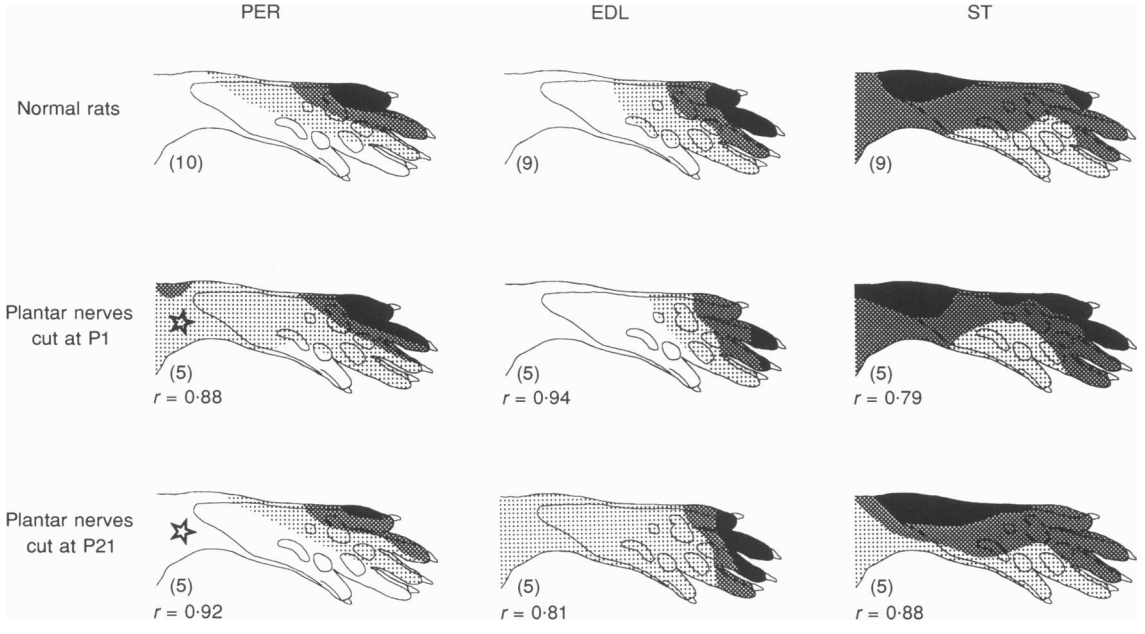

Figure 2. Receptive fields of three hindlimb muscles obtained in normal, P1-lesioned and P21-lesioned rats with thermal (CO_2 laser) stimulation

Mean receptive fields (see Methods) of PER, EDL and ST obtained using thermal (CO_2 laser) stimulation (intensity twice reflex threshold). Conventions as in Fig. 1.

mechano- and thermonociceptive receptive fields exhibited aberrant foci on the skin overlying the metatarsal bones in three of five rats lesioned at P21 (Fig. 3). Such anomalies were not seen in rats lesioned at P1. Also, unlike normal rats and rats lesioned at P1, the distribution of sensitivity differed between the mean mechano- and thermonociceptive receptive fields of EDL.

Responses elicited by stimulation of the skin overlying the neuroma

With the exception of mechanonociceptive reflexes of ST in P1-lesioned rats (which were increased to 320% of control, $P < 0{\cdot}001$), the magnitude of mechano- and thermonociceptive reflexes of PER, EDL and ST evoked by stimulation of the skin covering the transection neuroma (location indicated by ☆ in Figs 1 and 2; middle and bottom left) in rats lesioned at P1 or P21 were not significantly different from normal.

Temporal characteristics and magnitude of the reflexes

For EDL and PER, no statistically significant difference of mean onset latency or peak response latency of CO_2 laser-evoked reflexes was found in P1-lesioned rats (PER, $n = 5$; EDL, $n = 5$) or P21-lesioned rats (PER, $n = 5$; EDL, $n = 5$) as compared with normal rats (PER, $n = 10$; EDL, $n = 9$) (Fig. 4). For ST, there was a small but significant reduction of mean onset latency (84% of normal onset latency, $P < 0{\cdot}05$), but not of peak response latency, in P1-lesioned rats ($n = 5$) as compared with normal rats ($n = 9$). There was no change of onset or peak response latency of reflexes of ST in P21-lesioned rats ($n = 5$).

In rats lesioned at P1, PER and EDL reflexes evoked by noxious mechanical stimulation (PER, $n = 7$; EDL, $n = 7$) were reduced to 50% as compared with those in normal rats (PER, $n = 12$; EDL, $n = 9$) ($P < 0{\cdot}05$). The magnitude of reflexes of ST evoked by noxious pinch in P1-lesioned rats ($n = 7$) did not differ significantly from that in normal rats ($n = 8$). Neither in PER, nor in EDL, was there any significant difference in the mean magnitude of reflexes evoked by CO_2 laser in P1-lesioned rats (PER, $n = 5$; EDL, $n = 5$) as compared with normal rats. By contrast, in ST there was a significant increase of CO_2 laser-evoked reflexes (190% of normal values, $P < 0{\cdot}01$) in P1-lesioned rats ($n = 5$) as compared with normal rats.

In rats lesioned at P21, there was a considerable reduction of reflex magnitude in both PER ($n = 5$ rats) and EDL ($n = 5$ rats) for noxious pinch (to 15–30% of normal values; PER, $P < 0{\cdot}001$; EDL, $P < 0{\cdot}05$), and also for CO_2 laser stimulation (to 20–25% of normal values; PER, $P < 0{\cdot}001$; EDL, $P < 0{\cdot}01$). There was no significant difference between the magnitude of either noxious pinch ($n = 5$ rats) or CO_2 laser-evoked ($n = 5$ rats) reflexes of ST in P21-lesioned rats as compared with normal rats.

Cutaneous innervation patterns

Rats lesioned at P1. After acute selective transection of the plantar nerves in rats lesioned at P1, clear reflexes could be evoked by noxious pinch ($n = 4$ rats) and CO_2 laser ($n = 3$ rats) stimulation of virtually all sites tested on the plantar skin (Figs 5 and 6; middle left). By contrast, after an identical nerve lesion in normal animals ($n = 4$ rats) reflexes could not be evoked from a large portion of the plantar skin (Figs 5 and 6; top left).

After acute transection of the saphenous, sural and common peroneal nerves, sparing the plantar nerves ($n = 3$), the magnitude of the reflexes was very low (about 10% of the magnitude of reflexes evoked under identical conditions in normal rats) and responses were evoked mainly from the proximal part of the plantar surface of the foot (Figs 5 and 6; middle right). After a subsequent transection of the plantar nerves no reflexes at all could be evoked from the

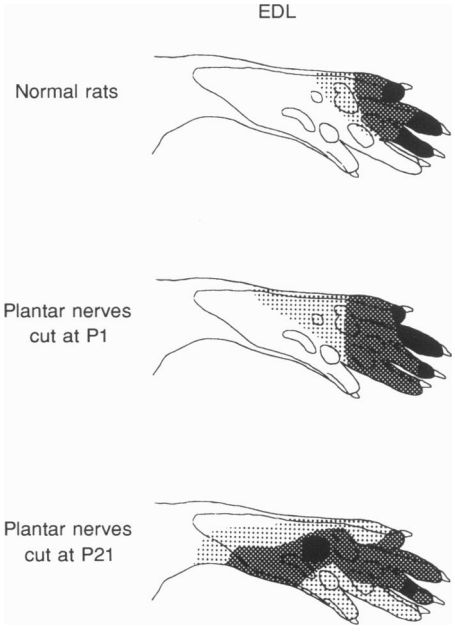

Figure 3. Samples of EDL receptive fields obtained using noxious pinch stimulation in single normal, P1-lesioned and P21-lesioned rats

Low, medium and high density of dots indicate areas of the skin from which the evoked reflexes were 0–30%, 30–70% and 70–100% of maximal response, respectively.

plantar surface of the foot, indicating that these remaining reflexes were dependent on transmission via the regenerated plantar nerves.

In order to obtain information concerning changes in the size of the innervation fields of adjacent nerves the contribution from the common peroneal nerve to the reflexes was assessed in two rats lesioned at P1. After acute transection of the plantar, sural and saphenous nerves, reflexes could be evoked by both pinch and CO_2 laser stimulation of a large portion of the distal part of the plantar surface of the foot (Fig. 7; bottom left). After the same nerve lesions in normal animals ($n = 2$; Fig. 7; top left), reflexes were only evoked from parts of digits 2 and 3 and distal digit 5 (and the hindpaw dorsum, not shown), and only by pinch stimulation, never by CO_2 laser.

In the two P1-lesioned rats in which the common peroneal nerve had been selectively spared, the superficial peroneal nerve was subsequently cut, thus sparing only the deep peroneal nerve. Clear reflexes could then still be evoked by both noxious mechanical and CO_2 laser stimulation of the distal pads and digits 2 and 3 (Fig. 7; bottom right). These reflexes were abolished after acute transection of the deep peroneal nerve. By contrast, in normal rats the contribution from the deep peroneal nerve to reflexes evoked from the plantar surface of the foot was negligible ($n = 2$; Fig. 7; top right)

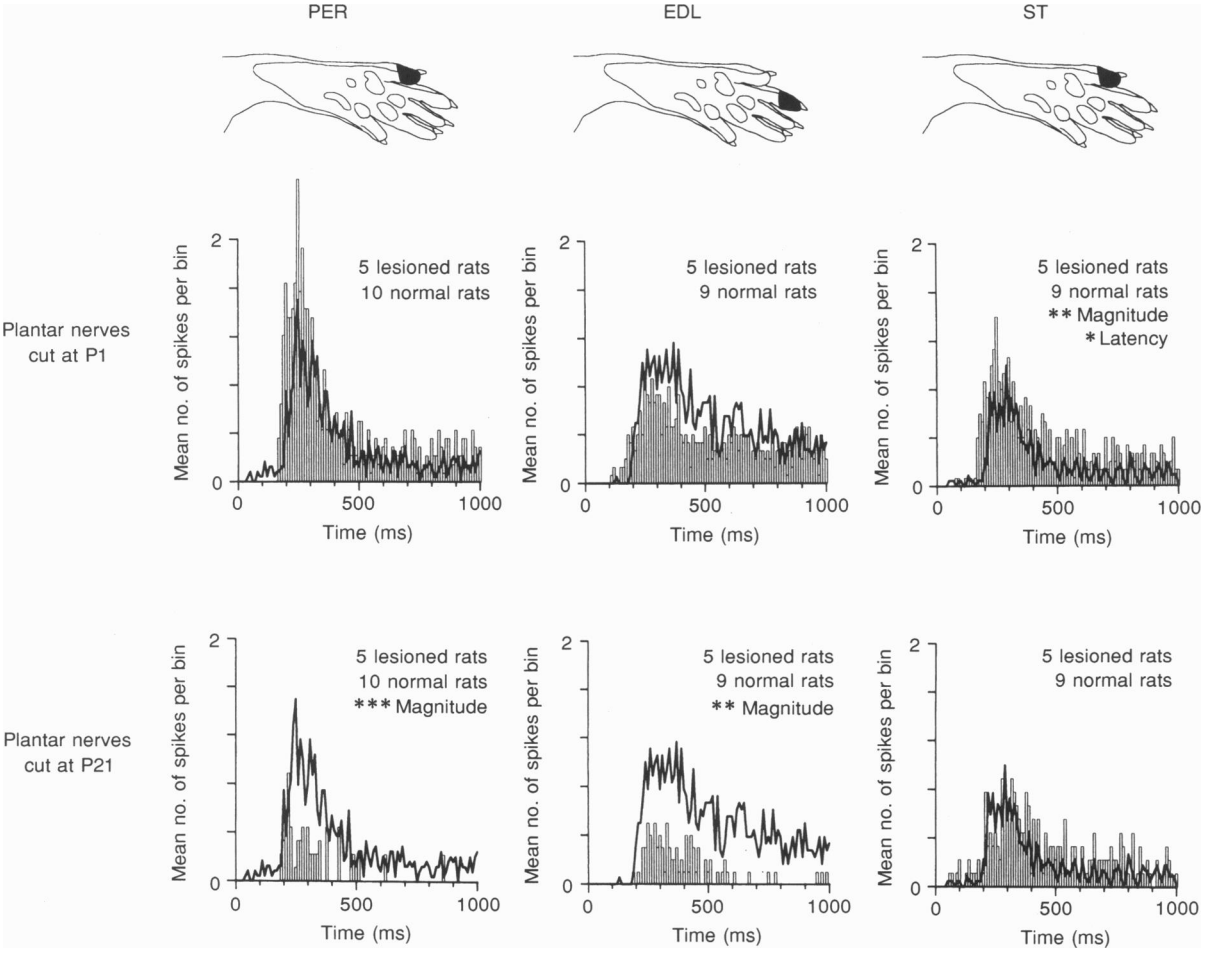

Figure 4. Mean post-stimulus time histograms (PSTHs) of CO_2 laser-evoked reflex responses in three hindlimb muscles in normal, P1-lesioned and P21-lesioned rats

PSTHs of CO_2 laser-evoked reflexes in the investigated muscles in P1- and P21-lesioned rats are shown as bars. Corresponding PSTHs of reflex responses evoked in the same muscles in normal adult rats under identical experimental conditions are superimposed (thick lines). The skin area from which maximal responses are evoked in the respective muscle in normal adult rats (indicated above each column) was stimulated (intensity twice reflex threshold). For each group of muscles two reflex responses were sampled from each rat. The number of lesioned and normal rats is indicated in each PSTH. Statistically significant latency and magnitude differences between the lesioned and normal rats are indicated. * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$; *** $P < 0{\cdot}001$. Bin time, 10 ms.

In three other rats lesioned at P1 only the deep peroneal and the regenerated plantar nerves were spared. In these animals reflexes were evoked from the proximal paw, the distal pads and from digits 2 and 3 by both noxious mechanical and CO_2 laser stimulation. These findings were thus clearly in line with the above described contributions from the deep peroneal and plantar nerves in the P1-lesioned group.

Rats lesioned at P21. The portion of the plantar surface of the foot which was rendered unresponsive to stimulation after acute transection of the plantar nerves ($n = 2$ rats; see above) was smaller in the P21-lesioned rats (Figs 5 and 6; bottom left) than in normal rats ($n = 4$) (Figs 5 and 6; top left). After transection of the saphenous, sural and common peroneal nerves, sparing the plantar nerves ($n = 2$ rats; see above), reflexes were evoked from a larger portion of the plantar surface of the foot in the P21-lesioned rats (Figs 5 and 6; bottom right) than in the P1-lesioned rats (Figs 5 and 6; middle right). Furthermore, the magnitude of reflexes evoked from the plantar surface of the foot was higher than in P1-lesioned rats under the same conditions (about 35% and 10% of responses evoked after selective sparing of the plantar nerves in normal rats, respectively). These reflexes were abolished after subsequent acute transection of the regenerated plantar nerves. The areas from which the common peroneal nerve or its branches provided reflex input were not examined in detail in the P21-lesioned rats.

Appearance of the regenerated plantar nerve in the P1 and P21 group

There was a pronounced difference in the gross appearance of the plantar nerves in rats lesioned at P1 as compared with rats lesioned at P21. Whereas in the P1 group the diameter of the regenerated

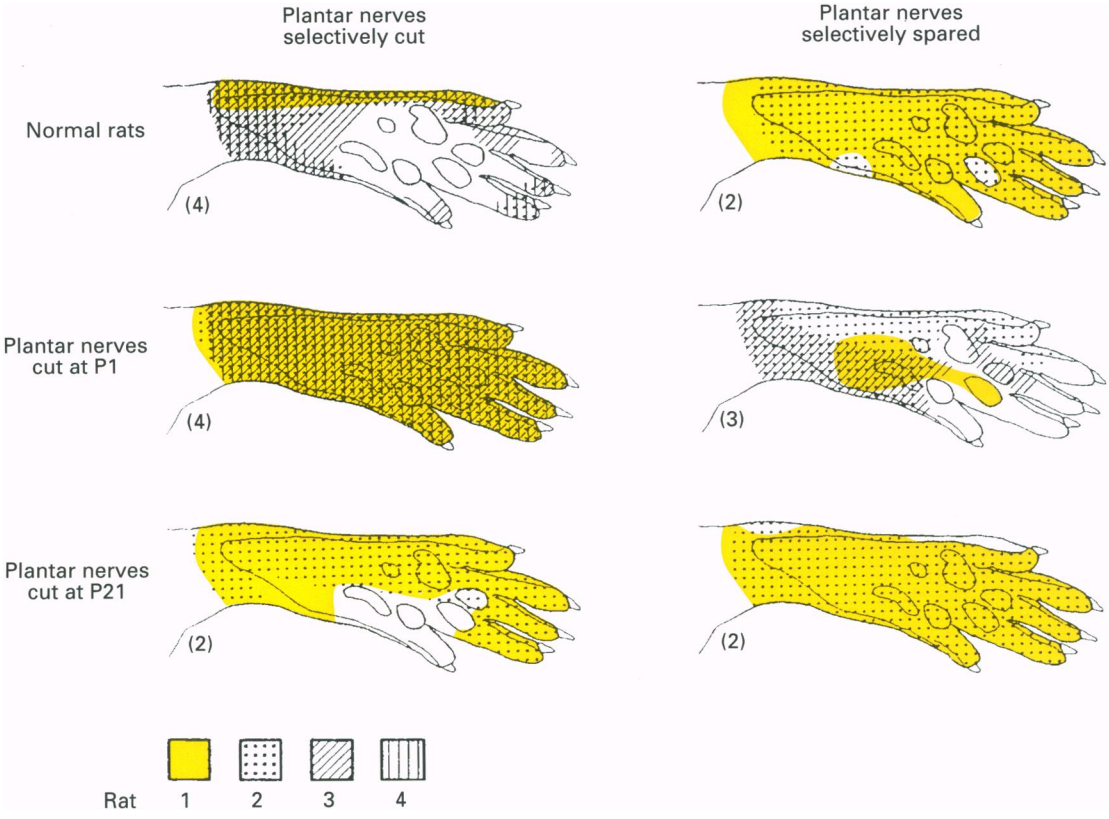

Figure 5. Areas of the plantar hindpaw skin from which EMG recorded reflex activity could be evoked by noxious mechanical stimulation in any of PER, EDL or ST after acute selective transection or selective sparing of the plantar nerves

The plantar nerves were either selectively transected (left column), or selectively spared (i.e. the saphenous, sural and common peroneal nerves were transected; right column) in normal adult rats (upper row) and in rats subjected to transection of the plantar nerves at P1 or P21 (middle and bottom row, respectively). Marked areas indicate portions of the hindpaw skin from which reflex activity in any of PER, EDL or ST could be evoked. Results from single experiments are indicated superimposed as follows: rat 1, yellow area; rat 2, dots; rat 3, hatched lines; and rat 4, vertical lines. No reflexes were evoked in any of the muscles on stimulation of white (unmarked) areas. Note (i) the limited extent of the area from which no responses could be evoked after acute transection of the plantar nerves in the P1-lesioned rats compared with normal rats; and (ii) the relatively large area from which no responses could be evoked after selective sparing of the plantar nerves in P1-lesioned rats compared with normal rats and P21-lesioned rats.

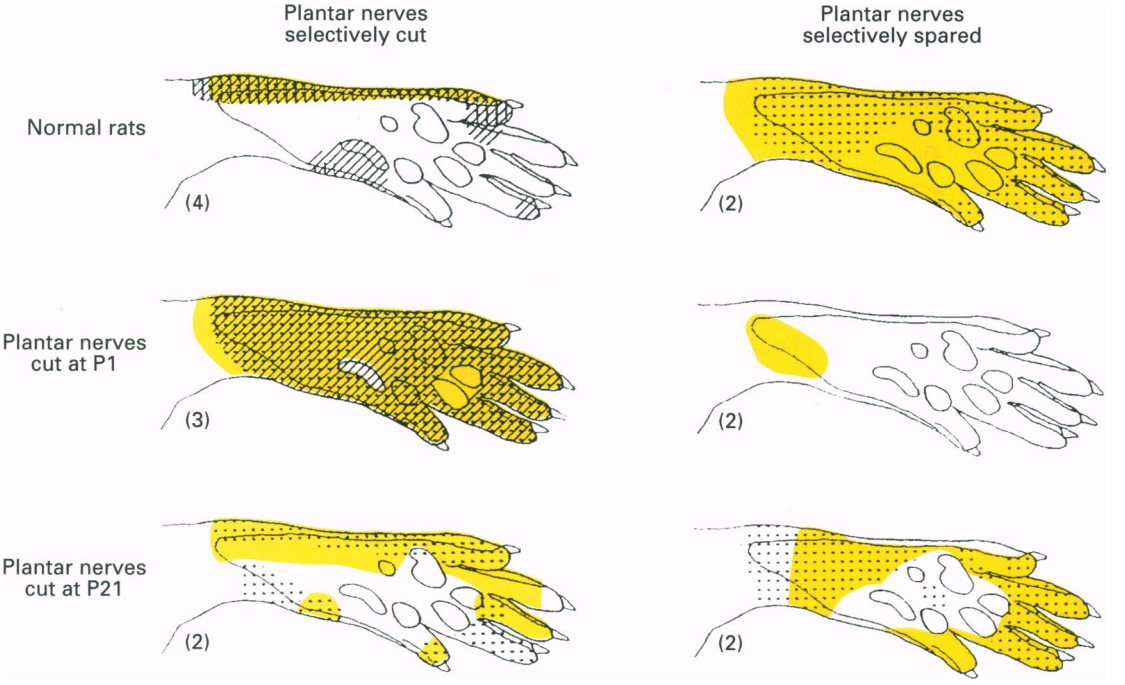

Figure 6. Areas of the plantar hindpaw skin from which EMG recorded reflex activity could be evoked by thermal (CO_2 laser) stimulation in any of PER, EDL or ST after acute selective transection or selective sparing of the plantar nerves

Conventions and acute manipulations of innervation as in Fig. 5.

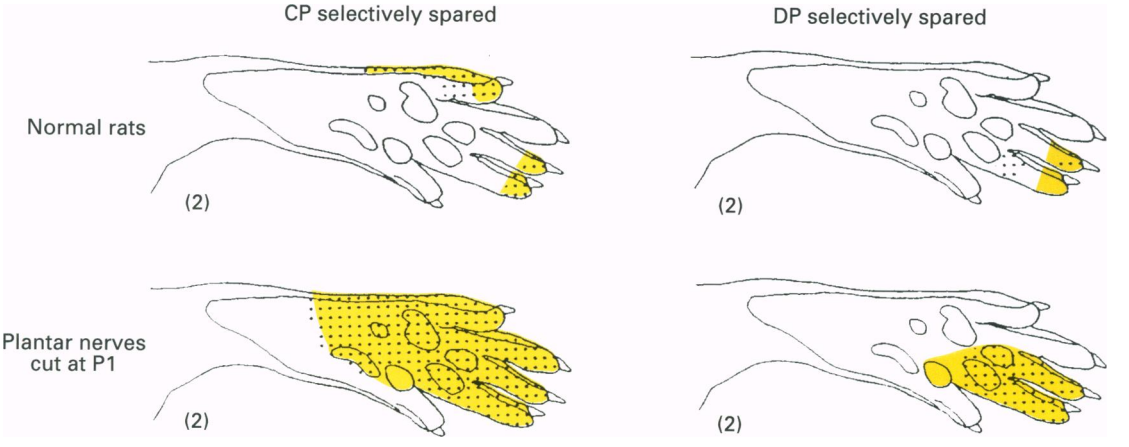

Figure 7. Areas of the plantar hindpaw skin from which EMG recorded reflex activity could be evoked by noxious mechanical stimulation in any of PER, EDL or ST after acute selective transection of hindlimb nerves sparing either the common peroneal nerve or the deep peroneal nerve

The common peroneal nerve (CP) was selectively spared by acute transection of the plantar, saphenous and sural nerves (left column). The deep peroneal nerve (DP) was selectively spared by additional acute transection of the superficial peroneal nerve (right column). Upper row, normal adult rats; bottom row, adult rats subjected to transection of the plantar nerves at P1. Noxious mechanical stimulation. Conventions as in Fig. 5. Note that compared with normal rats, there was a marked expansion of the area from which reflex responses were evoked after selective sparing of CP or DP in the P1-lesioned rats.

plantar nerves was about one-third of that on the contralateral side, there was only a minor reduction in rats lesioned at P21. In all lesioned animals a large transection neuroma had formed at the lesion site (the location of the neuroma is indicated by ☆ in Figs 1 and 2, middle and bottom left).

DISCUSSION

The present study demonstrates that despite the substantial rearrangement of functional peripheral innervation which is induced by neonatal transection of the plantar nerves, thermo- and mechanonociceptive withdrawal reflexes evoked from the plantar surface of the foot attain a near normal spatial organization. These findings confirm and extend previous demonstrations of recovery of nociceptive reflex responses from skin areas initially rendered anaesthetic by early peripheral nerve transection (Devor *et al.* 1979; Mills, Nurse & Diamond, 1989) and add to the accumulating amount of data demonstrating plasticity of the developing nervous system (see O'Leary, Ruff & Dyck, 1994).

Furthermore, when the peripheral innervation was altered after the third postnatal week, the adaptation of the withdrawal reflexes appeared less precise, suggesting age-related decrement of the capacity for functional reorganization. Peripheral and central changes possibly underlying the functional adaptation of nociceptive withdrawal reflexes after a nerve lesion will be discussed below.

On the changes of functional hindpaw innervation

In the present study the skin areas on the plantar surface of the hindpaw from which withdrawal reflexes could be evoked were delineated in adult rats after acute transection of peripheral nerves innervating the hindpaw (Greene, 1963). Whereas this method provides information on *functional innervation* with respect to input to the withdrawal reflexes, it does not necessarily reveal the entire extent of the morphologically detectable innervation fields. Thus, although the mappings were made in a spinal preparation with a relatively high reflex excitability, the possibility of hidden non-functional inputs, which became functional after nerve section, cannot be ruled out on the basis of the present data. Nevertheless, the delimited functional innervation fields for the plantar and the common peroneal nerves found in normal rats in the present study are consistent with previous descriptions of the peripheral innervation fields of these hindlimb nerves obtained by morphological techniques (Swett & Woolf, 1985; Molander & Grant, 1986) and by studies of Evans Blue extravasation evoked by C-fibre stimulation of single hindlimb nerves (Wiesenfeld-Hallin, 1988).

P1-lesioned rats. It is known that following peripheral nerve lesions in neonatal rats there is a massive death of the axotomized dorsal root ganglion cells (Bondok & Sansone, 1984). Accordingly, in the present study, the regenerated plantar nerves of rats lesioned at P1 were found to be very small and their contribution to the functional innervation of the plantar skin was essentially abolished. Instead, the functional innervation fields of other peripheral nerves had expanded into the plantar skin. In the case of the superficial and deep branches of the common peroneal nerve this expansion was dramatic. Hence, a profound change of the innervation of the plantar skin occurs after neonatal transection of the plantar nerves. These results complement previous studies reporting that after sciatic nerve transection the peripheral terminal field of the saphenous nerve expands into the plantar skin (Devor *et al.* 1979; Kinnman & Aldskogius, 1986).

P21-lesioned rats. After acute section of the saphenous, sural and common peroneal nerves, sparing the regenerated plantar nerves only, clear reflex responses were evoked from almost the entire plantar surface of the foot in P21-lesioned rats. This indicates a better recovery of the function of the plantar nerves in the P21-lesioned rats than in the P1-lesioned rats, and is consistent with the reported finding that the axotomy-induced loss of dorsal root ganglion cells is reduced after P10 (Devor, Govrin-Lippmann, Frank & Raber, 1985). Collateral sprouting from nearby nerves apparently also contributed to the innervation of the plantar surface of the foot since the portion of the hindpaw rendered unresponsive to noxious stimulation after acute selective transection of the plantar nerves in the P21 group was smaller than in normal rats.

On the reflex time course and magnitude

With the exception of a small reduction in latency of the thermoreceptive reflexes of ST in the P1 group, there was no significant change of the time course (onset and peak latencies) of the reflexes evoked in the nerve-lesioned groups. This indicates that the reflexes in the nerve-lesioned rats were evoked by the same type of afferent fibres as in normal rats. Furthermore, as sprouted afferent fibres (Shortland & Fitzgerald, 1991), but not regenerated fibres (Horch & Lisney, 1981), appear to restore normal conduction velocities it may be that the early phase of the withdrawal reflexes in both nerve-lesioned groups were evoked by sprouted fibres.

In adult P1-lesioned rats, the receptive fields of PER, EDL, and ST had a near-normal spatial organization, irrespective of whether thermal or mechanical stimulation was used. However, while the magnitude of *thermonociceptive* reflexes was normal for PER and EDL, and even increased for ST, the magnitude of mechanonociceptive PER and EDL reflexes was reduced compared with normal, while being normal for ST. Thus, the restoration of the reflex magnitude differed between the different muscles and between the different stimulation types used. That mechanonociceptive reflexes tended to be less restored than thermonociceptive reflexes may suggest that mechanonociceptive fibres have a lower capacity than thermonociceptive fibres to emit

functional peripheral and/or central collateral sprouts in response to denervation of adjacent skin.

The differences in the restoration of reflexes of ST as compared to that of PER and EDL may be related to the position of the central terminals of the hindlimb nerves relative to the position of reflex interneurones within the respective reflex pathways. Interneurones encoding withdrawal reflex activity in PER and EDL appear to be preferentially located medially in L4 and L5, and those encoding ST reflexes more laterally in the same segments (Schouenborg *et al.* 1995). The central terminals of sural, common peroneal and saphenous afferent fibres, which are found laterally to the termination of the plantar nerves in these segments (Schouenborg, 1984; Swett & Woolf, 1985; Molander & Grant, 1986), are therefore in close proximity to ST encoding interneurones. It is known that central reorganization mainly occurs longitudinally (Snow & Wilson, 1989). Therefore, if compensatory central reorganization is a prerequisite for a functionally adapted reflex organization to emerge in the face of aberrant peripheral innervation patterns, functional recovery of ST reflexes may be relatively favoured in our experimental protocol.

On the mechanisms tuning the withdrawal reflex circuitry

The present study demonstrates that reflexes evoked from the plantar surface of the foot attain a near-normal spatial organization despite profound alterations of the innervation of the plantar skin induced at birth. This result indicates that the establishment of an appropriate reflex organization is not genetically 'hardwired', but rather at least partly under epigenetic influence.

In a preceding study we found that the nociceptive withdrawal reflex system is functionally unadapted at birth and attains its adult-like task specific organization over the first 2–3 postnatal weeks (Holmberg & Schouenborg, 1996). During this time inappropriate reflex connections become depressed, or eliminated, and appropriate reflex connections appear to be strengthened. Moreover, the finding that the withdrawal movement pattern of a single muscle is 'imprinted' on the receptive field of the muscle in this process, prompted us to postulate that the sensory feedback ensuing on contraction of single muscles is instrumental for tuning the withdrawal reflex circuitry during normal development (Schouenborg & Weng, 1994; Holmberg & Schouenborg, 1996). Such a mechanism could also underlie the functional adaptation to altered cutaneous innervation patterns. If the cutaneous feedback that follows contraction in a muscle were used to set the distribution of reflex gain, the input strength of a primary afferent to a given reflex module would be determined by the location of the afferent's receptive field in relation to the movement pattern ensuing on contraction of the module's effector muscle. Primary afferent fibres acquiring new receptive fields through collateral sprouting and establishing functional contact with the reflex interneurones, would be equally well suited to provide cutaneous feedback as the 'original' afferents. Further support for this hypothesis comes from our recent finding that neonatal tendon transfer, which alters the withdrawal movement ensuing on contraction in a single muscle, leads to a corresponding alteration of the distribution of sensitivity within the receptive field of the manipulated muscle (Schouenborg, Holmberg, Yu & Weng, 1994).

Age-dependent capacity for functional adaptation

Some observations in the present study may suggest that the capacity for adaptation to novel innervation patterns decreases with age. Firstly, the reflex magnitudes of PER and EDL appeared closer to normal in rats lesioned at P1 than in rats lesioned at P21. Secondly, receptive fields of EDL with aberrant foci on the skin overlying the metatarsal bones were found in three of five rats lesioned at P21, but never in rats lesioned at P1. To some extent these differences may be due to a marked age-dependent reduction in the ability of afferent fibres to undergo peripheral and central sprouting following nerve injury (Fitzgerald, 1985; Snow & Wilson, 1989; Kinnman, Aldskogius, Wiesenfeld-Hallin & Johansson, 1991). Nevertheless, a recovery of apparently normal contiguous receptive fields of dorsal horn neurones after sciatic nerve transection in the adult rat has been reported (Lewin, Mckintosh & McMahon, 1994). To what extent such plasticity can restore appropriate sensorimotor transformation of spinal reflexes after nerve lesions in adult rats remains to be determined.

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